

BREEDING PIERCE'S DISEASE RESISTANT TABLE AND RAISIN GRAPES AND THE DEVELOPMENT OF MARKERS FOR ADDITIONAL SOURCES OF RESISTANCE

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ABSTRACT

The first BC5 *Vitis arizonica* crosses (98.5% *V. vinifera*) were made in 2011 to continue creating germplasm with Pierce's disease (PD) resistance and high fruit quality. A total of 29 seedless x seedless crosses to develop BC3, BC4, and BC5 *V. arizonica* x *V. vinifera* families were made. The crosses consisted of 61,611 emasculations and produced 5,430 ovules and 877 (16.2%) embryos for PD resistance. An example of increased fruit quality is the selection for propagation in first stage production trials of six BC4 table, 8 BC4 and 3 BC3 raisin grape selections. One of the table grape selections is good enough to put in a 25 vine production trial. A total of 18 crosses to combine PM and PD resistance were made and consisted of 40,179 emasculations, 3,571 ovules and 651 embryos. The use of molecular markers to select for PD resistance and greenhouse screening to select for powdery mildew (PM) resistance allows selection of these two types of resistance before planting to the field. Two table and five raisin grapes were propagated into first stage production trials from 31 PD plus PM resistant seedlings. PD resistance of over 38 parents and selections from *V. arizonica* populations have been tested in greenhouse tests to insure resistance continues to co-segregate with markers. Two hundred and forty-three seedlings of the BD5-117 mapping family, with PD resistance different than *V. arizonica*, have been greenhouse tested. A preliminary rough molecular map based on 70 SSR primers and 144 seedlings indicates a major QTL for PD resistance on linkage group 2.

LAYPERSON SUMMARY

Although Pierce's disease (PD) has existed in California since the late 1800s, the introduction of the glassy-winged sharpshooter to California in the late 1990's significantly increased the spread and damage caused by PD. A collaborative breeding program was started in 2000 to develop PD resistant table and raisin grapes with high fruit quality comparable to cultivars existing in markets today. Sixth generation (BC5) crosses to produce quality table and raisin grapes with *Vitis arizonica* source of PD resistance were made for the first time this year. These families will have high fruit quality as they consist of 98.5% *V. vinifera*. An example of increased fruit quality is the selection for propagation in first stage production trials of six BC4 table, 8 BC4 and 3 BC3 raisin grape selections. These selections show some commercial potential with one table selections showing enough promise for inclusion in 25 vine cultural production trial. Crosses to combine powdery mildew (PM) and PD resistance were also made. The use of molecular markers to select for PD resistance and greenhouse screening to select for powdery mildew PM resistance allows selection before planting seedlings to the field. Two table and five raisin grapes with PD plus PM resistance were selected and propagated in first stage production trials. PD resistance of over 38 parents and selections from *V. arizonica* populations have been tested in greenhouse tests to insure the molecular markers for PD resistance continue to function properly. Two hundred and forty-three seedlings of the BD5-117 mapping family, which has PD resistance different than *V. arizonica*, have been greenhouse tested. A preliminary rough molecular map based on 70 SSR primers and 144 seedlings indicates a major QTL for PD resistance on linkage group 2. This means that selected molecular markers from linkage group 2 might be useful as markers to select resistance from BD5-117 source of resistance. This collaborative research between USDA/ARS, Parlier and University of California, Davis has the unique opportunity to develop high quality PD resistant table and raisin grape cultivars for the California grape industry where PD could restrict the use of conventional table and raisin grape cultivars.

INTRODUCTION

Pierce's disease (PD) has existed in California since the late 1800s when it caused an epidemic in Anaheim. A number of vectors for PD already exist in California, and they account for the spread and occurrence of the disease. The introduction of the glassy-winged sharpshooter to California in the 1990's significantly increased the spread and damage caused by PD. Other vectors exist outside California and are always a threat. All of California's commercially grown table and raisin grape cultivars are susceptible to PD. An effective way to combat PD and its vectors is to develop PD resistant cultivars so that PD epidemics or new vectors can be easily dealt with. PD resistance exists in a number of *Vitis* species and in *Muscadinia*. PD resistance has been introgressed into table grape cultivars in the southeastern United States, but fruit quality is inferior to *V. vinifera* table grape cultivars grown in California. No PD resistant raisin grape cultivars exist. Greenhouse screening techniques have been improved to expedite the selection of resistant individuals (Krivanek et al. 2005, Krivanek and Walker 2005). Molecular markers have also been identified that make selection of PD resistant individuals from *V. arizonica* even quicker (Krivanek et al. 2006). The USDA, ARS grape breeding program at Parlier, CA has developed elite table and raisin grape cultivars and germplasm with high fruit quality. Embryo rescue procedures for culturing seedless grapes are being

used to quickly introgress the seedless trait with PD resistance (Emershad et al. 1989). This collaborative research gives the unique opportunity to develop high quality PD resistant table and raisin grape cultivars for the California grape industry.

OBJECTIVES

1. Develop PD resistant table and raisin grape germplasm/cultivars with fruit quality equivalent to present day cultivars.
2. Develop molecular markers for Xf/PD resistance in a family (SEUS) other than *V. arizonica*.

RESULTS AND DISCUSSION

Objective 1

Twenty-nine of 33 crosses using *V. arizonica* source of resistance made in 2011 were successful, consisting of 61,611 emasculations, and produced 5,866 berries, 5,430 ovules, and 877 embryos (16.2% embryos/ovules) (**Table 1**). The majority of these were BC4 and the first BC5 crosses. The seedlings obtained from these crosses should have high fruit quality as they now have 97 to 98.5% *V. vinifera* in their background. An additional 18 crosses to pyramid PD (*V. arizonica*) resistance with powdery mildew (PM) (*V. romanetii*) resistance consisting of 40,179 emasculations produced 3,772 berries, 3,571 ovules and 651 (18.2%) embryos (**Table 1**). Ten and five seeded crosses made in 2011 produced 646 seed for PD resistance and 1,190 seed for PD plus PM resistance respectively (**Table 2**). Leaves from all 2010 *V. arizonica* PdR1 plants were taken when seedlings were still in test tubes starting in November, 2010. They were tested for resistance with molecular markers for the PdR1 locus on chromosome 14. Results for 11 BC3 and 13 BC4 seedless x seedless families is shown in **Table 3**. A total of 213 individuals were tested with SSR markers and 159 showed markers on both sides of the PdR1 region. A total of 73 individuals (46% of those showing markers) were resistant and 86 plants had susceptible markers. This is very similar to the ratio of resistant and susceptible plants obtained for over 1,600 F1, BC1, BC2 table and raisin seedlings reported by Riaz et al. 2009. The susceptible and recombinant individuals were discarded, making more efficient use of greenhouse and field space. From the crosses made in 2010 which combined PD resistance from *V. arizonica* with PM resistance from *V. romanetii*, 79 seedlings were screened with molecular markers for PD resistance and in the greenhouse for PM resistance. The segregation ratios are shown in **Table 4** and they are similar to the expected ratios for segregation of PD and PM as single dominant genes. Inoculation of plants with *Xylella fastidiosa* (Xf) in the Greenhouse (method of Krivanek et al. 2005, Krivanek and Walker 2005) was done to determine resistance of 38 selected individuals from *V. arizonica* (**Table 5**) of which 14 are resistant to date. These seedlings represent the best table and raisin selections that have been used as parents or planted in production trials. Greenhouse testing is absolutely necessary to make the final decision about resistance of individual selections. The highest level of resistance is being obtained from *V. arizonica* and its use will continue to be emphasized.

In 2011 five BC3 table grape selections were treated with 20 ppm gibberellic acid (GA) at berry set to determine berry response. In all cases the berries increased in weight, diameter and length (**Table 6**). GA treated berries from all selections were similar in size or larger than Thompson Seedless produced by standard commercial practices at the ARS Parlier research station. The treatments used for Thompson Seedless were 15ppm GA x 2 bloom sprays; 20ppm GA bump spray; 60ppm GA x 2 size sprays; girdle and tip. The selection 08-5001-34 (Fig. 1A) had larger berries than both samples of Thompson Seedless and had only one application of GA. 08-5001-34 has very acceptable fruit quality. The majority of 800 PD resistant BC3 and BC4 *V. arizonica* seedlings planted in 2009 and 2010 fruited and were evaluated. From these seedlings, the number of selections good enough for propagation into the first stage production trials is: 6 BC4 table selections, 8 BC4 and 3 BC3 raisin selections. Fruiting characteristics of these selections are shown in **Table 7**. One of the table grape selections, 09-5013-118 (**Figure 1B**), is good enough for inclusion in the 25 vine advanced cultural production trial. One table grape selection with PD plus PM resistance was also selected for propagation. In addition the following selections were made for use as parents: 3 BC4, 9 BC3 and 1 BC1 table grape; and 6 BC4 raisin grapes. In 2011, 6 table and 8 raisin PD resistant selections and 2 table and 5 raisin PD plus PM resistant selections were planted in production trials. Half of the planting (four of 8 replications) of 12 selections at the USDA ARS research station, Weslaco, Texas were inoculated with Xf on July 15 with the help of David Appel, Texas A&M University. Each plant was inoculated in twice. Leaf samples will be collected this fall to determine Xf levels.

Objective 2

The PD resistant grape selection BD5-117 from Florida was hybridized with the seedless table grape selection C33-30 to create the mapping population of over 500 individuals. Fruit samples are being taken from all seedlings to have three years data for berry weight and seed/seed trace weight as an indication of fruit quality. Flower type is also being recorded. Greenhouse testing for PD resistance has been accomplished on 243 individuals, with 112 rated clearly resistant or clearly susceptible. One hundred twenty-two individuals are being evaluated this year (**Table 5**). The 70 polymorphic markers tested on 144 individuals and greenhouse PD resistance evaluations were run in JoinMap which indicated a major QTL on linkage group 2. Forty additional polymorphic primers have been tested on the 144 seedlings for further refinement of the rough framework map. In 2011, nine seedless x seedless and three seeded by seedless BC1 crosses were made for table grape improvement using resistant F1 selections from BD5-117 (**Table 1 and 2**). A total of 22 and 8 clusters were pollinated for these crosses and resulted in 304 embryos and 526 seed from seedless x seedless and seeded x seedless crosses respectively.

CONCLUSIONS

Additional families for the development of PD resistant seedless table and raisin grape cultivars are being produced. Emphasis was placed on making the first BC5 and additional BC4 *V. arizonica* PD resistant families. These families will have high fruit quality as they consist of 97 – 98.5% *V. vinifera*. The use of molecular markers has simplified and sped up the identification of PD resistant individuals from *V. arizonica*. Seedless table and raisin grape selections with PD resistance and improved fruit quality have been made in both BC4 and BC3 *V. arizonica* and F1 BD5-117 families. For example, six new table and 11 new raisin PD resistant and one new table PD plus PM resistant selections were made from BC4 and BC3 *V. arizonica* families and will be propagated for production trials. Two hundred and forty-three seedlings from the BD5-117 family to develop a frame-work map for this source of PD resistance have been evaluated in the greenhouse for PD resistance. Initial mapping indicated a major QTL for resistance on linkage group 2 in the BD5-117 family. The development of PD resistant table and raisin grape cultivars will make it possible to keep the grape industry viable in PD infested areas. Molecular markers will greatly aid the selection of PD resistant individuals from SEUS populations.

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Table 1. 2011 table and raisin grape PD resistant seedless crosses that set fruit and the number of ovules and embryos produced.

Female	Male	Type	No. Emas- culations	No. berries Opened	No. Ovules	No. Embryos
89-0908 <i>V. rupestris</i> x <i>V. arizonica</i>						
04-5015-5	C 61-123	Table BC2	1201	60	46	21
04-5514-144	Y125- 39--05	Table BC2	1 bag	185	315	36
06-5503-107	Y129-161--05	Table BC4	619	90	92	2
Y152-128--08	08-5001-20	Table BC4	3797	27	47	25
06-5501-229	Y536- 53	Table BC4	2702	408	270	28
05-5501-69	B 26-120	Table BC4	5 bags	197	5	0
06-5503-126	Y537-131--06	Table BC3	694	227	136	30
06-5501-423	05-5502-15	Table BC3	1737	212	257	65
06-5501-423	05-5501-27	Table BC3	3064	80	108	20
06-5503-126	C 61-123	Table BC4	1814	458	650	114
06-5503-107	Y142- 54--09	Table BC4	2029	152	166	2
06-5503-121	06-5503-121	Table BC4	1755	200	63	2
08-5001-41	Y525- 60--07	Table BC4	4243	356	500	86
09-5013-013	Y530- 5--07	Table BC5	5 bags	450	500	122
09-5013-067	Y530- 5--07	Table BC5	3015	21	0	0
A 51- 36	05-5551-116	Raisin BC4	2744	422	341	53
07-5052-033	A 63- 85	Raisin BC4	4202	550	483	57
A 51- 36	07-5061-072	Raisin BC4	2437	340	182	68
09-5057-048	B 82- 43	Raisin BC5	5 bags	455	500	26
09-5064-006	B 82- 43	Raisin BC5	4 bags	3	0	0
A 63- 85	09-5056-012	Raisin BC5	2113	43	81	7
A 63- 85	09-5064-038	Raisin BC5	2502	218	268	46

Table 1 continued. 2011 table and raisin grape PD resistant seedless crosses that set fruit and the number of ovules and embryos produced.

Female	Male	Type	No. Emas- culations	No. berries Opened	No. Ovules	No. Embryos
A 63- 85	09-5072-24	Raisin BC5	2552	102	33	0
B 82- 43	09-5056-012	Raisin BC5	2550	135	0	0
B 82- 43	09-5063-023	Raisin BC5	2561	27	22	1
Y143- 26	09-5063-023	Raisin BC5	2489	53	14	2
Y143- 26	09-5064-016	Raisin BC5	1301	70	11	3
Y143- 26	09-5072-024	Raisin BC5	2315	25	23	4
Y144-132--04	09-5074-002	Raisin BC5	2636	300	317	57
Total			61,611	5,866	5,430	877
PM resistance combined with 89-0908 <i>V. rupestris</i> x <i>V. arizonica</i>						
05-5501-69	Y312-187--06	Table BC3	3 bags ^z	143	50	2
05-5501-69	Y313-157--08	Table BC3	4 bags	47	36	1
06-5501-229	Y313-191--08	Table BC3	86	14	7	1
06-5501-238	Y308-311--06	Table BC3	1395	200	75	87
Y315-400--04	05-5501-27	Table BC3	2098	73	122	41
Y520- 71--07	08-5001-20	Table BC3	1957	190	198	20
08-6003-002	Y152- 84--08	Table BC5	3453	191	200	38
07-5052-033	Y308-312--06	Raisin BC4	2723	381	487	72
07-5052-033	Y308-329--06	Raisin BC4	2100	195	205	19
A 49- 82	08-6053-020	Raisin BC4	2500	21	32	2
A 51- 36	08-6053-012	Raisin BC4	4374	189	159	23
A 63- 85	08-6053-020	Raisin BC4	3646	214	116	10
08-6052-003	A 51- 60	Raisin BC4	1998	550	500	71
08-6053-028	A 51- 43	Raisin BC4	2627	304	386	73
08-6053-028	A 63- 85	Raisin BC4	2866	520	500	87
B 82- 43	08-6053-012	Raisin BC4	2571	300	265	62
B 82- 43	08-6053-020	Raisin BC4	2481	200	195	36
09-5013-013	Y308-329--06	Raisin BC5	5 bags	40	38	6
Total			40,179	3,772	3,571	651
PD resistance from BD5-117						
03-5003-103	Y540-193--05	Table BC1	3 bags	241	400	75
03-5003-108	Y140- 54--08	Table BC1	3 bags	225	400	17
03-5003-103	Y534-101--06	Table BC1	2 bags	284	472	86
03-5003-108	Y537-168--06	Table BC1	2 bags	115	156	5
03-5003-090	Y534- 91--06	Table BC1	3 bags	126	90	15
03-5003-110	Y538-181--06	Table BC1	2 bags	27	4	0
03-5003-090	Y537- 32--06	Table BC1	2 bags	180	107	20
03-5003-090	03-5003-082	Table BC1	2 bags	211	104	12
03-5003-103	03-5003-082	Table BC1	3 bags	353	500	74
Total			22 bags	1,762	2,233	304

^z Clusters bagged because flowers are female and do not need emasculation.

Table 2. 2011 table and raisin grape PD resistant seeded crosses and the number of seeds produced.

Female	Male	Type	No. Emas- culations	No. seed
89-0908 <i>V. rupestris</i> x <i>V. arizonica</i>				
04-5002-18	Y525- 60--07	Table BC1	388	96
04-5514-144	05-5501-27	Table BC2x3	3 bags ^z	13
05-5501-26	Y127-111--05	Table BC3	8 bags	21
05-5501-06	Y152-128--08	Table BC3	5 bags	235
07-5060-134	Y525- 60--07	Table BC3	2 bags	69
07-5060-134	Y540-193--05	Table BC3	3 bags	153
07-5060-134	05-5501-27	Table BC3x3	3 bags	1

Table 2 continued. 2011 table and raisin grape PD resistant seeded crosses and the number of seeds produced.

Female	Male	Type	No. Emas- culations	No. seed
07-5060-134	C 61-123	Table BC3	1 bags	30
07-5060-134	C 75- 4	Table BC3	1 bags	28
Total			29 bags +388	646
PM resistance combined with PD resistance				
07-5060-134	Y308-148--06	Table BC3	1 bags	315
05-5501-57	06-3551-226	Table BC3	1076	231
Y308- 14	Y520- 73--07	Table BC3	2354	269
Y308- 39	Y520- 73--07	Table BC3	1162	184
07-5052-61	Y308-311--06	Table BC4	2999	191
Total			7,591	1,190
PD resistance from BD5-117				
03-5003-052	Y537-168--06	Table BC1	3 bags	172
03-5003-052	Y139-139--04	Table BC1	3 bags	246
03-5003-052	Y131-181--05	Table BC1	2 bags	108
Total			8 bags	526

^z Clusters bagged because flowers are female and do not need emasculation.

Table 3. Determination of seedling resistance based on PdR1 molecular markers for all 89-0908 families made in 2010.

Family	Type Cross	No. Resistant ^a	No. Susceptible ^b	No. Recombinant ^c	No data ^d	Off Types	Total
10-5004	Table BC4	3	1				4
10-5005	Table BC3	5	2	1			8
10-5006	Table BC3					1	1
10-5007	Table BC3	3	1	3		2	8
10-5008	Table BC3	1	9	1		21	32
10-5009	Table BC3	1	1				2
10-5013	Table BC4					3	3
10-5015	Table BC4	5	1			1	7
10-5052	Raisin BC4	1					1
10-5054	Raisin BC4	2	2				4
10-5063	Raisin BC4	2				1	3
10-5065	Raisin BC4	4	1		1		6
10-5073	Raisin BC4	2	3				5
10-5074	Raisin BC4	6	4	1			11
10-5076	Raisin BC4	2	14	1			20
10-6001	Table BC3	8	17	1			26
10-6002	Table BC3		3				3
10-6003	Table BC3	2	3				5
10-6005	Table BC3	1					1
10-6007	Table BC4					4	4
10-6051	Raisin BC3	1					1
10-6053	Raisin BC3	22	23	2		5	52
10-6056	Raisin BC4	1				1	2
10-6058	Raisin BC4	1	1			2	4
Total		73	86	10	1	41	213

^aResistant = marker on both sides of *PdR1* region.

^bSusceptible = no *PdR1* markers.

^cRecombinant= genotypes that amplified with one *PdR1* marker.

^dNo data = genotypes that failed to amplify properly.

^e%= Number of seedlings in each category / total number of seedlings showing the proper markers.

Table 4. Segregation of PD and PM resistance in seedlings from 2010 crosses combining resistance from *V. arizonica* and *V. romanetti*, respectively.

		PD resistance from <i>V. arizonica</i>		
		PD Resistant Obs./Exp.	PD Susceptible Obs./Exp.	Total Obs./Exp.
PM resistance from <i>V. romanetti</i>	PM Resistant Obs./Exp.	18/20	28/20	46/40
	PM Susceptible Obs./Exp.	17/20	16/20	33/40
	Total Obs./Exp.	35/40	44/40	79/80

Table 5. Results of greenhouse test for determination of PD reaction in 2011.

Population	Resistance Source	Testing Compete		In greenhouse test
		No. tested	No. resistant	For evaluation by December
BD5-117 map	BD5-117	30	3	89
Arizonica	PdR1	22	14	16
Other PD	SEUS	2	0	6
TOTAL		54	17	111

Table 6. Berry size of PD resistant table grape selections treated with 20ppm gibberellic acid at berry set or no treatment (NT).

Name	Treatment	mean ber. Wt. (g)	mean ber. Dia. (mm)	mean ber. Len. (mm)
08-5001-34	NT	7.5	22.05	24.5
08-5001-34	GA	11.7	23.52	33.32
08-5001-21	NT	4.4	18.13	19.6
08-5001-21	GA	7.1	21.07	24.5
08-5001-38	NT	3.7	16.66	17.64
08-5001-38	GA	6.4	20.58	24.01
08-5001-47	NT	3.6	16.17	17.64
08-5001-47	GA	6.0	20.09	21.56
TS ARS Plot	GA	6.8	19.2	31.2
TS commercial	GA	10.2	22.0	36.0

Table 7. New PD resistant grape selections made and evaluated 2011.

Name	genera- tion	color	trace	berry size	crop	cluster size	berry set	firm- ness	status	comment
Table PD <i>V. arizonica</i> resistance										
09-5013-070	BC4	W8	10	13	2	2	4	8	prop	P1 GH test
09-5013-075	BC4	R8	10	14	4	4	5	3	prop	P1 GH test
09-5013-118	BC4	R7	7	13	3	5	5	6	prop*	GH test
09-5013-122	BC4	W7	10	12	3	4	4	5	prop	GH test
09-5013-125	BC4	W8	5	12	2	4	6	7	prop	GH test
09-5064-022	BC4	W7	5	14	4	5	5	4	prop	P1 GH test
PD +PM resistance										
08-6002-089	BC3	W6	6	13	2	5	4	2	prop	P1 GH test

Table 7 continued. New PD resistant grape selections made and evaluated 2011.

Name	generation	trace	berry size	crop	cluster size	berry set	flavor	status	Type	comment
Raisin PD <i>V. arizonica</i> resistance										
09-5066-063	BC4	7	midget	1	1		neutral	prop	DOV	GH test
09-5066-097	BC4	8	TS					prop	DOV	P1
09-5068-023	BC4	9	TS	1	1		muscat	prop	DOV	GH test
09-5070-101/015	BC4	10	TS	6	5	5	neutral	prop	TD	GH test
09-5070-015/5071-007	BC4	10	TS	6	5	5	neutral	prop	TD	GH test
09-5063-018	BC4	7	TS	3	4	5	neutral	prop	DOV	GH test
09-5063-021	BC4	8	TS	2	4	4	neutral	prop	DOV	GH test
09-5066-039	BC4	7	midget	4	3	5	neutral	prop	DOV	GH test
09-5056-089	BC3	10	TS	6	5	5	neutral	prop	TD	GH test
08-5056-059	BC3	8	midget	6	4	5	neutral	prop	TD	GH test
08-5056-072	BC3	10	midget	5	4	5	neutral	prop*	TD	GH test
08-5054-047	BC1	10	TS	4	4	4	neutral	prop	TD	GH test

Trace: 10=none, 9=Thompson Seedless (TS) size, 7= Fiesta size, 6=Flame Seedless size

Berry size = 1/16" for table grapes and Thompson Seedless (TS) or midget size for raisins.

Crop: 9=v. heavy, 7=heavy, 5=average, 3=light, 1=v. light. Cluster size: 9=>2.5 lb, 7=2 lb, 5=1 lb, 3=0.5 lb, 1=widow size.

Berry set: 9=v. tight, 7=tight, 5=average, 3=loose, 1=v. loose. Firmness: 9=v. firm, 7=firm, 5=average, 3=soft, 1=v. soft.

Prop = propagate in first 2 vine plot; prop* = propagate table grape in 25 vine or raisin in 7 vine production trial.

DOV = natural dry on the vine type. TD = tray dried. GH test = test resistant reaction to *Xylella* in greenhouse.



Figure 1. A. Fruit of BC3 PD resistant table grape selection 08-5001-34 treated with 20ppm size GA. B. Natural fruit of BC4 PD resistant table grape selection 09-5013-118, first crop on seedling.